OPEN ACCESS Freely available online

Journal of Clinical Trials

Research Article

# Impact of Probiotics on Gut Microbiome Bifidobacterium Relative Abundance: First Do No Harm

**Daniels Jordan*, Papoutsis Andreas, Barrows Brad, Hazan Sabine**

*ProgenaBiome, Ventura, California, United States*

## ABSTRACT

**Background:** Several reports have raised safety concerns regarding the use of probiotics. To address these concerns, this study examined the relative abundance (proportion of the microbiome made up of a particular taxa) and normalized read counts (number of times a particular microbe was identified) of Bifidobacteria in the gut microbiome of healthy subjects participating in an ongoing study on the microbiome. Bifidobacteria is a critically important constituent of the human microbiome and plays roles in digestion, gut immunity, and cancer prevention.

**Methods:** Fecal samples were analyzed using next-generation sequencing to evaluate composition and relative abundance of bacterial phyla through species level in each subject`s microbiome. The primary outcomes of this subgroup analysis were relative abundance and normalized read count of genus Bifidobacteria in subjects who took unregulated probiotics, regulated probiotics, or no probiotics.

**Results:** The relative abundance and normalized read count of Bifidobacteria were significantly lower in the microbiome of subjects who took unregulated probiotics (n=15) than in the microbiomes of both those who took regulated probiotics (n=12, P=0.0002) and no probiotics (n=13, P=0.0483) (0.18 vs. 9.59 vs. 5.66 relative abundance).

**Discussion:** Subjects taking unregulated probiotics had a significantly lower relative abundance of Bifidobacteria, which could potentially have a detrimental impact on health. Next-generation sequencing could be a useful tool to guide decisions on the appropriate use of probiotics based on dysbiosis.

**Keywords:** *Bifidobacterium*; Gut microbiome; Probiotics; Whole genome sequencing; Shotgun sequencing

## INTRODUCTION

Human host-bacterial symbiosis has been extensively investigated, particularly in the last two decades. Bacteria reside in all parts of our body, with the largest reservoir being the gastrointestinal tract, collectively referred to as the gut microbiome [1]. There is mounting evidence of the critical role of the gut microbiome in human health and disease, which has led to a soaring demand for probiotics and supplements [2]. In 2016, the global probiotics market was valued at approximately $36.6 billion USD, with an anticipated value of $64 billion USD by 2023 [3].

The World Health Organization (WHO) has defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host"[4]. Probiotics provide health benefits by modulating gut microbiota. And although some probiotic bacteria are "generally recognized as safe," several recent reports have raised safety concerns over their use [5]. Concerns raised include risk of systemic infection, metabolic disruption, and immune system overstimulation as well as potential horizontal gene transfer between bacteria (facilitating antibiotic resistance). Cases of bacterial translocation leading to sepsis have been reported following probiotic administration [5].

**Correspondence to:** Daniels Jordan, ProgenaBiome, Ventura, California, United States, E-mail: jordan@progenabiome.com

**Received date:** July 28, 2021; **Accepted date:** August 10, 2021; **Published date:** August 17, 2021

**Citation:** Jordan D, Andreas P, Brad B, Sabine H (2021) Impact of Probiotics on Gut Microbiome Bifidobacterium Relative Abundance: First Do No Harm. J Clin Trials. 11:473.

Recently, several researchers raised safety concerns regarding the use of probiotics [5,6], particularly in at risk populations, including neonates and those with clinical conditions such as malignancies, autoimmune conditions, diabetes mellitus, and post-organ transplantation status. Some probiotic strains may adversely affect those with compromised immunity and potentially facilitate life- threatening infections including pneumonia, endocarditis, and sepsis [6,7].

There is a substantial body of "evidence" advocating the benefits of probiotic supplements. However, the quality of evidence, as well as the quality of the production of probiotic supplements are matters of concern. In a systematic review of the effects of *Lactobacillus casei* or *Bifidobacterium lactis* on a healthy population, 16 studies were evaluated to assess health benefit claims [8]. Of 47 studies listed on the corporate website, all were funded by the company and only 12 overlapped with those meeting inclusion criteria for the systematic review. Only seven of the 12 demonstrated some positive effect following consumption of probiotic-enriched milk products. And none of these seven actually demonstrated a clinical benefit to use of the product; rather, they extrapolated from laboratory values. The three pertaining to *L. casei* received a recommendation grade of D for relying primarily on circumstantial evidence and opinion. In contrast, the four pertaining to *B. lactis* were of sufficient scientific rigor to merit a recommendation grade of [9,10]. These results suggest that there may be inadequate scientific evidence to support the health claims made for many probiotic products.

The quality of probiotics is also of concern, largely due to the absence of supplement regulation. In a national survey of commercial probiotic food supplements in Italy, of 25 samples that claimed *B. bifidum* was present, none contained the live bacterium and the non-viable bacterium was only sporadically present [11]. More importantly, some of these supplements contained toxin-producing species of bacteria including Bacillus cereus. Another study that evaluated 16 probiotic products found the contents of only one brand to match its label claims. And even within that particular brand, inter- and intra-lot variation was noted [12]. In many cases the products contained little or no viable bacteria. A third study involving 26 probiotic products found instances of misidentification of the bacteria at both the species (27 incorrectly identified) and genus levels (19 incorrectly identified) [13,14]. This misidentification is clinically significant. As quoted by Rijkers et al in 2011, "Stig Bengmark has made this very clear in his statement that the (genetic) difference between one probiotic bacterium and the other is larger than the difference between a man and a goldfish." This is illustrated by a study which compared the activity of two different Lactobacillus strains (*Lactobacillus salivarius CECT5713* and *Lactobacillus fermentum CECT5716*) in mice. Despite the similarities between the strains, *L. fermentum* was found to be immunostimulatory, whereas *L. salivarius* was found to have an anti-inflammatory response [15]. When dietary supplement production facilities were inspected in 2019, over half (305/598) were issued Form 483 for violations of manufacturing standards and safety. The most commonly cited violation categor–accounting for 25% of violations–was failure to adequately describe the final product, including identity, purity, and strength. Other violations included an absence of written quality control procedures, failure to follow written procedures, and incomplete batch records [16]. Poor quality control was identified as the main factor leading to non-compliance. Given the staggering lack of quality control checks in the probiotic industry, choosing an appropriate probiotic supplement is challenging.

Because 1) probiotics can adversely modify gut flora, 2) clinical studies on their effects are of questionable quality, and 3) probiotics lack clear endorsement or approval by regulatory authorities or agencies, choosing a suitable probiotic poses a considerable challenge. Determining the composition of an individual's microbiome prior to the start of therapy, would allow targeted rather than "empiric" therapy. Such a personalized approach could serve as a safety measure for preventing possible infection and/or pathologic dysbiosis, which could mitigate associated morbidity and mortality [17]. Next-Generation Sequencing (NGS) of the gut microbiome could become an important tool for identifying gut dysbiosis.

In this study we use next-generation sequencing of the gut microbiome to determine composition and relative abundance in association with the clinical characteristics of patients, following probiotic administration. We describe the results of a subgroup analysis performed to identify changes in microbiota in terms of fecal Bifidobacterium counts in patients taking probiotics dichotomized on regulation or lack thereof compared to those who did not take probiotics.

## MATERIALS AND METHODS

### Study design and participant selection

This was a prospective, longitudinal descriptive study that used shotgun NGS to characterize the gut bacterial microbiome. This study conducted at ProgenaBiome commenced in April 2019 and is currently ongoing. The protocol was approved by Ethical & Independent Review Board and is being conducted in accordance with the applicable requirements outlined in the United States Food and Drug Administration (FDA) Code of Federal Regulations (CFR) Title 21 and International Conference on Harmonisation (ICH) E6 Good Clinical Practice guidelines. All participants signed written informed consent.

During their regular medical visit, men and women of any age who desired to know the composition of their gut microbiome were enrolled in the study. In this study, both healthy volunteers, as well as patients with 34 pre-specified health conditions (Table 1) under examination were included. For the subset analysis, only data from healthy participants were included. Exclusion criteria included history of recent antibiotic use, bariatric surgery, total colectomy with ileorectal anastomosis, proctocolectomy, postoperative stoma, ostomy, ileoanal anastomosis, total parenteral nutrition, or participation in an experimental drug investigation within 12 weeks of our study commencement.

Subjects were selected for this subgroup analysis if they had no known medical issues and had used a single probiotic supplement for at least six weeks prior to sample collection–hereafter termed "healthy."

DNA extraction and sequencing: Fecal samples were collected using either a Zymo Research DNA/RNA Shield Fecal Collection Tube (Zymo Research, Freiburg, Germany) or a OMNIgene Gut Collection Vial (DNA Genotek, Ottawa, CA). Following fecal

sample collection, individual subject DNA was extracted and purified using the Qiagen PowerFecal Pro DNA extraction kit (Qiagen, Hilden, Germany). The isolated DNA was then quantified with the Quantus Fluorometer with the QuantFluor ONE dsDNA kit (Promega, Madison, WI, USA). After DNA quantification, the DNA was normalized, and sample libraries were prepared utilizing shotgun methodology with Illumina's Nextera Flex kit. Sample libraries were then normalized and pooled for sequencing on Illumina's NextSeq 550 System. Following completion of the NextSeq run, the raw.bcl data were streamed to Illumina's BaseSpace cloud for conversion to FASTQ files. The FASTQ files were then pushed through the bioinformatics metagenomics pipeline, with subject specific readouts profiling each subject's unique microbiome.

## Study outcomes

All subjects whose gut microbiome was sequenced for this study were volunteers for the larger study on the characterization of the gut microbiome. This subset was selected based on overall health and lack of gastrointestinal symptoms. The primary outcome of this subgroup analysis was Bifidobacterium relative abundance in subjects who used unregulated probiotics, regulated probiotics, or no probiotics. "Unregulated" probiotics were defined as over-the-counter branded pills that are not regulated by the FDA. Probiotics were classified as "Regulated" are FDA approved.

## Analysis

Sequencing results were examined for both relative abundance and normalized read counts, both generated by the bioinformatics pipeline. Relative abundance indicates what percentage of the sample is made up of a particular taxa of microbes. Normalized read count indicates how many times a particular microbe was identified in a sample, using calculations to correct for the difference in genome size between organisms. Figure 1 was generated using *Bifidobacteria* relative abundance values from the bioinformatics pipeline. Figure 2 was generated by identifying the 17 most prevalent genera across the groups.

## Statistical analysis

Continuous variables were presented as mean with Standard Deviation (SD) or median (Interquartile Range [IQR]). ANOVA was performed to identify the difference between the groups. Categorical variables were reported as frequencies and percentages and compared using Pearson $\chi 2$ or Fisher's exact test as applicable. A value of $P<0.05$ was considered statistically significant. The data were analyzed by SPSS version 11.5 for Windows (SPSS Inc., Chicago, IL, USA). The subjects in this analysis comprised the 40 for whom details on the type of probiotics used was available.

## RESULTS

Of the 40 subjects included in this study, 15 subjects used unregulated probiotics; 12 subjects used regulated probiotics; and 13, used none. The mean age of the subjects was 46.8 years, and a majority (27/40, [67.5%]) were women. All were considered healthy, with the primary difference being the *Bifidobacteria* in their microbiome. While subject age, sex, diet, and blood type were recorded, no correlations were noted probiotics taken or not taken, and the relative abundance of between the relative abundance of *Bifidobacteria* and these variables.

Of the remaining 16 most prevalent genera, only *Faecalibacterium*, *Ruminococcus*, *Eubacterium*, *Alistipes*, and *Parabacteroides* were found to be statistically significant (Table 1).

| Organism | p value | Description of Difference |
|---|---|---|
| *Bifidobacteria* | 0.0001 | Lowest in Unregulated group |
| *Facaelibacterium* | 0.01747 | Lowest in Unregulated group |
| *Bactroides* | 0.391733 | Not Significant |
| *Clostridium* | 0.897584 | Not Significant |
| *Ruminococcus* | 0.003704 | Lowest in Unregulated group |
| *Eubacterium* | 0.027509 | Lowest in Unregulated group |
| *Alistipes* | 0.019949 | Lowest in Regulated group, highest in Unregulated group |
| *Blautia* | 0.727962 | Not Significant |
| *Dorea* | 0.911675 | Not Significant |
| *Coprococcus* | 0.677123 | Not Significant |
| *Roseburia* | 0.701591 | Not Significant |
| *Subdoligranulum* | 0.491463 | Not Significant |
| *Oscillobacter* | 0.662898 | Not Significant |
| *Akkermansia* | 0.143468 | Not Significant |
| *Prevotella* | 0.694261 | Not Significant |
| *Parabacteroides* | 0.024904 | Highest in unregulated group |
| *Collinsella* | 0.528406 | Not Significant |

**Table 1:** Statistical findings for 17 most prevalent genera found in the microbiome of subjects. The p value for *Bifidobacteria* is at least one order of magnitude smaller than the other significant genera.

The significance of *Bifidobacteria* was at least one order of magnitude greater than the other significant genera. As such that became the focus of this study. Shannon Diversity Index was not found to differ significantly between groups.

Figure 1 illustrates the relative abundance of *Bifidobacteria* for each subject by probiotic group. Subjects taking unregulated probiotics had a lower mean relative abundance of *Bifidobacteria* in their microbiome than both subjects taking regulated probiotics and subjects who did not take probiotics. The mean *Bifidobacteria* relative abundance was 0.1[0.04-0.4], 4.9 [0.1-26.6] and 4.9 [1.8-23.57] in the unregulated probiotics, no probiotics, and regulated probiotics groups, respectively ($p<0.0001$).

Figure 2 illustrates the normalized read counts of the 17 most prevalent genera identified by shotgun sequencing, as determined by normalized read counts.

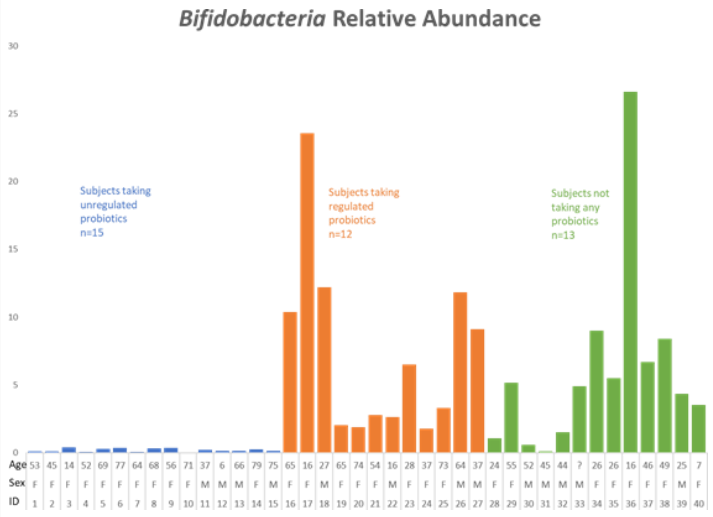


**Figure 1:** *Bifidobacteria* relative abundance in subjects who consumed unregulated probiotics (blue), subjects who consumed regulated probiotics (orange), and subjects who did not take any probiotic (green).

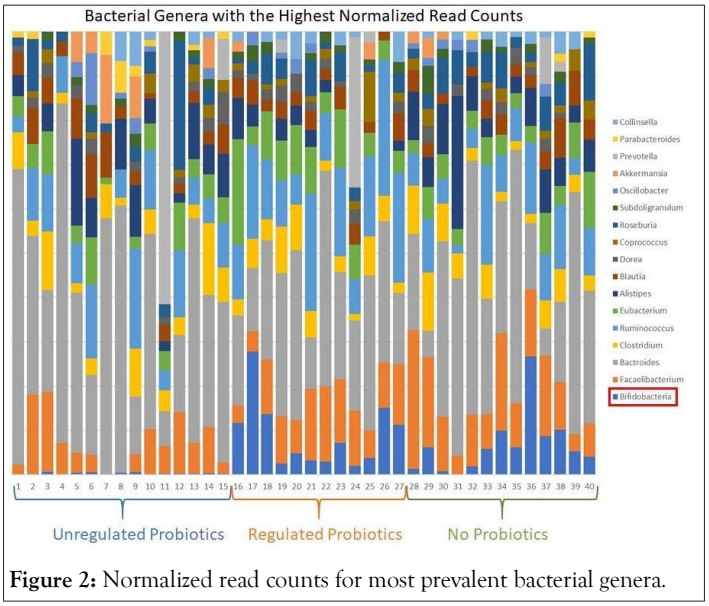


**Figure 2:** Normalized read counts for most prevalent bacterial genera.

## DISCUSSION

Our study demonstrates the possibility that unregulated probiotic supplements may negatively alter the microbiome of patients with respect to maintaining healthy levels of Bifidobacteria. Subjects who took unregulated probiotics had lower Bifidobacteria levels than both subjects taking regulated probiotics and those taking no probiotics.

These results were an incidental finding from a larger study on the microbiome and will be followed by a randomized controlled clinical trial with probiotics-naïve subjects so that a baseline microbiome profile can be established. While Bifidobacteria will be examined in this study, a full microbiome analysis will also be performed. The probiotics used by subjects in this trial will also be tested for contents and viability.

We hypothesize that the presence of nonviable Bifidobacteria might have a detrimental impact on the relative abundance of viable Bifidobacteria by stimulating macrophages to regard the remaining Bifidobacteria as "other," resulting in an inflammatory response by the host. Future studies could examine the levels of inflammatory markers in subjects undergoing microbiome analysis [17-20].

The relevance of lower relative abundance of Bifidobacteria in the human gut microbiome is that Bifidobacteria play a crucial role in the nutrition of the host. These obligate anaerobes utilize the bifid shunt, or fructose-6-phosphate phosphoketolase pathway, to degrade monosaccharides such as glucose and fructose. The resulting ATP production is higher from this pathway than more traditional fermentative pathways [20]. They can also perform intercellular degradation of oligosaccharides which gives them a competitive advantage over other colonic bacteria such as Lactobacillus [20]. Additionally, the metabolic by-products and end-products help maintain the balance of the gut microbiome by feeding other bacteria involved in butyrate production, which helps with inflammation and prevention of cancer [20]. If our hypothesis regarding inflammatory response due to the presence of nonviable Bifidobacteria is correct, it could result in a positive feedback loop of inflammation.

The other significant genera certainly play important roles in the health of the host. Genera Faecalibacterium, Eubacterium, and Ruminococcus, found to be lower in the Unregulated group, are Short-Chain Fatty Acid (SCFA) butyrate producers. Reduced levels of SCFAs can induce inflammation through many pathways including reduced differentiation of naïve T cells into regulatory T cells (TREG), increased insulin resistance, and increased neutrophil chemotaxis. However, R. gnavus produces an inflammatory glucorhamnan polysaccharide which is recognized by Toll-Like Receptor 4 (TLR4) and induces dendritic cells to produce Tumor Necrosis Factor Alpha (TNFα), a potent inflammatory cytokine.

Genus Alistipes, identified in 2003, is not yet well understood. It is thought to be a producer of SCFA due to its role in the development of liver disease when depleted, although elevations in certain species have been positively correlated to increased systolic blood pressure in subjects with cardiovascular disease [21-26]. Alistipes was found to be lower in subjects who consumed Unregulated probiotics.

The role of Parabacteroides in the gut microbiome varies greatly by species. It has been implicated in the pathogenesis of a wide variety of conditions, from alopecia areata to decreased hippocampal function [26]. With all these critical roles of gut microbiota, manipulation of the relative abundance via prebiotics and probiotics is a logical, noninvasive step to take. Probiotics have several purported health benefits, including "supporting digestion, preventing and treating diarrhea, supporting oral health, improving a few mental health conditions, guaranteeing a healthy heart, relieving allergies and eczema, boosting immunity, taking care of belly fat, supporting vaginal health, treating irritable bowel syndrome, reducing blood pressure levels, preventing cancer, and alleviating respiratory disorder[s]" [27]. And while many of these claims are suspect, probiotics can be safe if used in suitable patients and have been manufactured with adequate quality controls. In this context, shifting physician focus toward characterizing the patient's gut microbiome prior to choosing a probiotic may be the best approach. Just as personalized oncology utilizes the genomic information of tumor cells to identify the most suitable chemotherapeutic agent products, personalized

probiotics could potentially be used to correct pathologic gut dysbiosis.

Our study demonstrates that the use of unregulated probiotics may reduce beneficial *Bifidobacteria* levels and highlights the potential benefit of gut microbiome sequencing prior to choosing a probiotic for a specific patient. Analysis and regulation of the probiotics themselves is also important to ensure that the beneficial, live microbes are present. In order to ensure that label claims are accurate, probiotic manufacturers need to conduct more frequent and thorough self-regulation. This must include having and following written quality control procedures, particularly with regard to species- and strain-level identification.

A major limitation of this study was the non-interventional study design. An ideal study on probiotics would compare the composition of a microbiome, including the relative abundance of microbes before and after probiotic consumption for several months. A small sample size is another major limitation of this study. The nature of whole genome sequencing prevents us from knowing whether the identified bacteria were live within the subjects' gut. We were also unable to test the probiotics themselves to determine whether the contents matched the label claims.

## CONCLUSION

In summary, the results of our study demonstrate the that the *Bifidobacteria* relative abundance was low in subjects using poor unregulated probiotics compared to those using good regulated or no probiotics. Evaluating the gut microbiome of an individual before and after administering/prescribing a probiotic may be a rational approach to determining the need for probiotic treatment, and if there is a need, to choosing a probiotic treatment and ensuring its benefit.

## DECLARATIONS

### Author contributions

Conceptualization: SH; AP: methodology; AP: analysis; AP: SH, JD; BB: investigation; SH: resources; SH: writing—original draft preparation; JD and AP: writing—review and editing; AP: SH; BB: visualization; JD: SH; AP: supervision; SH: project administration. All authors have read and agreed to the published version of the manuscript.

### Funding

This research received no external funding.

### Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethical & Independent Review.

### Informed consent statement

Informed consent was obtained from all subjects involved in the study.

### Acknowledgment

The authors wish to express gratitude to the late Dr. Sydney Finegold, without whom this research could not have been conducted.

## REFERENCES

1. Armour CR, Nayfach S, Pollard KS, Sharpton TJ. A metagenomic meta-analysis reveals functional signatures of health and disease in the human gut microbiome. M Systems. 2019;4:e00332-18.
2. Aureli P, Fiore A, Scalfaro C, Casale M, Franciosa G. National survey outcomes on commercial probiotic food supplements in Italy. Int J Food Microbiol. 2010;137:265-73.
3. Azad M, Kalam A, Sarker M, Li T, Yin J. Probiotic species in the modulation of gut microbiota: An overview. Biomed Res Int. 2018:9478630.
4. Cohen PA. Probiotic safety—no guarantees. JAMA Intern Med. 2018;12:1577-1578.
5. Hamilton-Miller JM, Shah S, Winkler JT. Public health issues arising from microbiological and labelling quality of foods and supplements containing probiotic microorganisms. Public Health Nutr. 1999;2: 223–229.
6. Díaz-Ropero MP. Two lactobacillus strains, isolated from breast milk, differently modulate the immune response. J Applied Microbiol. 2007;102:2.
7. Yang Y, Xin Y. Effect of a fermented milk containing *bifidobacterium lactis* DN-173010 on Chinese constipated women. World J Gastroenterol. 2008;14(40):6237.
8. Meance R, Séverine L. Recent advances in the use of functional foods: Effects of the commercial fermented milk with bifidobacterium animalis strain DN-173 010 and yoghurt strains on gut transit time in the elderly. Micr Ecol Health Dis. 2003;15:115–22.
9. Meance R, Séverine L. A fermented milk with a *bifidobacterium* probiotic strain DN-173 010 shortened oro-fecal gut transit time in elderly. Micr Ecol Health Dis. 2001;13(4):217–222.
10. Global market insights Inc. Probiotics market size to exceed USD 64 billion by 2023: Global market insights inc. pr newswire: News distribution, targeting and monitoring. 2018.
11. Kothari D, Patel S, Kim SK. Probiotic supplements might not be universally-effective and safe: A review. Biomed Pharmacother. 2019; 111:537-547.
12. Michael HL, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Land AKS, et al. Lactobacillus sepsis associated with probiotic therapy. Pediatrics 2005;115:178–181.
13. Lerner A, Shoenfeld Y, Matthias T. Probiotics: if it does not help it does not do any harm. Really? Microorganisms. 2019;4:104.
14. Lewis ZT, Shani G, Masarweh CF, Popovic M, Frese SA, David AS, et al. Validating *bifidobacterial* species and subspecies identity in commercial probiotic products. Pediatr Res. 2015;79:445–452.
15. Illanes M, Lorena R. Does the scientific evidence support the advertising claims made for products containing lactobacillus casei and *bifidobacterium lactis*? a systematic review. J Public Health. 2015;38:3.
16. Agrawal A. Clinical trial: The effects of a fermented milk product containing *bifidobacterium lactis* DN-173 010 on abdominal distension and gastrointestinal transit in irritable bowel syndrome with constipation." alimentary pharmacology &amp. Therapeutics. 2009;29(1):104–114.
17. Huys K, Geert R. Accuracy of species identity of commercial bacterial cultures intended for probiotic or nutritional use. Res Microbiol. 2006:157(9):803–810.

18. Rijkers G, De Vos W, Brummer R, Morelli L, Corthier G, Marteau P. Health benefits and health claims of probiotics: Bridging science and marketing. British J Nutr. 2011:106(9):1291-1296.
19. Josh L, Marchesi JR, Ravel J. FDA irked with basic dietary supplement manufacturing violations. Natural products INSIDER, 18 Dec. 2019, supplement-manufacturing- violations. The vocabulary of microbiome research: A proposal. Microbiome. 2015;3(1):31.
20. Vuyst L De. Summer meeting 2013: Growth and physiology of *Bifidobacteria*. J Applied Microbiol. 2013;116(3):477–491.
21. Michael J. 13 Amazing health benefits of probiotics. Natural food series. 2018.
22. Topol Eric J. Individualized medicine from prewomb to tomb. Cell, 2014;157(1):241–253.
23. Henke Matthew T et al. Ruminococcus Gnavus, a member of the human gut microbiome associated with crohn's disease, produces an inflammatory polysaccharide. Proceedings of the national academy of sciences. 2019;116(26):12672–12677.
24. Arghya M. Gut microbes from the phylogenetically diverse genus eubacterium and their various contributions to gut health. Gut Microbes. 2020;12(1):1802866.
25. Bianca JP. The genus alistipes: Gut bacteria with emerging implications to inflammation, cancer, and mental health. Frontiers Immunol. 2020:11.
26. Moreno-Arrones OM. Analysis of the gut microbiota in alopecia areata: Identification of bacterial biomarkers. J European Academy Dermatol Venereol. 2019;34(2): 400–405.
27. Noble EE, Olson CA, Davis E. Gut microbial taxa elevated by dietary sugar disrupt memory function. Transl Psychiatry. 2021;11: 194.